

What is claimed is:

1. A method of substantially preventing modification of a synthetic oligonucleotide or an oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group from the oligonucleotide or an oligonucleotide analog,  
5 comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising at least one acrylonitrile scavenger and an organic solvent selected from the group consisting of a haloalkane, an ester, an alcohol or dimethyl formamide under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group provided that  
10 when the acrylonitrile scavenge comprises a primary aliphatic amine or a primary aliphatic thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered.
2. A method of substantially preventing modification of a synthetic oligonucleotide or an oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group from the oligonucleotide or an oligonucleotide analog,  
15 comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with an aqueous basic solution having at least one acrylonitrile scavenger under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group provided that when the acrylonitrile scavenge  
20 comprises a primary aliphatic amine or a primary aliphatic thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered.
3. The method of Claim 2, further comprising the step of contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising an organic solvent and at least one acrylonitrile scavenger prior to

contacting the oligonucleotide or oligonucleotide analog with the aqueous basic solution.

4. The method of Claim 3, wherein the organic solvent is selected from the group consisting of pyridine, tetrahydrofuran, acetonitrile, a haloalkane, an esters, an alcohol and dimethyl formamide.
5. The method of Claim 4, wherein the acrylonitrile scavenger is *t*-butylamine and the organic solvent is pyridine or acetonitrile.
6. The method of Claim 2, wherein the  $\beta$ -cyanoethyl protecting groups are removed from a phosphate triester oligonucleotide.
7. The method of Claim 2, wherein the  $\beta$ -cyanoethyl protecting groups are removed from a phosphorothioate oligonucleotide analog.
8. The method of Claim 2, wherein the synthetic oligonucleotide or oligonucleotide analog is attached to a solid support by a covalent bond.
9. The method of Claim 8, wherein the solid support is controlled-pore glass, polystyrene or polyacrylamide.
10. The method of Claim 9, wherein the oligonucleotide or oligonucleotide analog is cleaved from the solid support by contact with a basic solution having at least one acrylonitrile scavenger.
11. The method of Claim 2, wherein the acrylonitrile scavenger is a substituted or unsubstituted sterically hindered aliphatic thiol group, a substituted or unsubstituted aromatic thiol, a substituted or unsubstituted aromatic hydroxyl

group, a substituted or unsubstituted secondary aliphatic amine, a substituted or unsubstituted sterically hindered primary aliphatic amine or a substituted or unsubstituted primary or secondary aromatic amine.

12. The method of Claim 11, wherein the acrylonitrile scavenger is *t*-butylamine.
- 5 13. The method of Claim 2, wherein the basic solution comprises an alkali metal hydroxide or an alkaline earth metal hydroxide.
14. The method of Claim 2, wherein the basic solution is an ammonium hydroxide solution.
- 10 15. The method of Claim 14, wherein the temperature of the ammonium hydroxide solution is about 20°C to about 100°C.
16. The method of Claim 15, wherein the temperature of the ammonium hydroxide solution is about 20°C to about 35°C.
17. The method of Claim 16, wherein the temperature of the ammonium hydroxide solution is about 25°C.
- 15 18. The method of Claim 14, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 0.5 hours to about 48 hours.
19. The method of Claim 18, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for  
20 about 0.5 hours to about 2 hours.

20. The method of Claim 2, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 5%.
21. The method of Claim 20, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 25%.
22. The method of Claim 21, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 50%.
23. The method of Claim 22, wherein all the nucleobases are thymine or guanine.
24. A method of substantially preventing modification of a synthetic oligonucleotide or oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group from the oligonucleotide or an oligonucleotide analog, comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising at least one acrylonitrile scavenger and an organic solvent selected from the group consisting of a haloalkane, an ester, an alcohol or dimethyl formamide under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group, provided that when the acrylonitrile scavenge comprises a primary aliphatic amine or a primary aliphatic thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered.

25. A method of substantially preventing modification of a synthetic oligonucleotide or oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group from the oligonucleotide or oligonucleotide analog, comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with an aqueous basic solution having at least one acrylonitrile scavenger under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group, provided that when the acrylonitrile scavenger comprises a primary aliphatic amine or a primary aliphatic thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered.
26. The method of Claim 25, further comprising the step of contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising an organic solvent and at least one acrylonitrile scavenger prior to contacting the oligonucleotide or oligonucleotide analog with the aqueous basic solution.
27. The method of Claim 26, wherein the organic solvent is selected from the group consisting of pyridine, tetrahydrofuran, acetonitrile, a haloalkane, an esters, an alcohol and dimethyl formamide.
28. The method of Claim 7, wherein the acrylonitrile scavenger is *t*-butylamine and the organic solvent is pyridine or acetonitrile.
29. The method of Claim 25, wherein a the  $\beta$ -cyanoethyl protecting groups and the nucleobase protecting groups are removed from a phosphate diester oligonucleotide.

30. The method of Claim 25, wherein a the  $\beta$ -cyanoethyl protecting groups and the nucleobase protecting groups are removed from a phosphorothioate oligonucleotide analog.
- 5 31. The method of Claim 25, wherein the synthetic oligonucleotide or oligonucleotide analog is attached to a solid support by a covalent bond.
32. The method of Claim 31, wherein the solid support is controlled-pore glass, polystyrene or poly(acrylamide).
- 10 33. The method of Claim 32, wherein the oligonucleotide or oligonucleotide analog is cleaved from the solid support by contact with a basic solution having at least one acrylonitrile scavenger.
- 15 34. The method of Claim 25, wherein the acrylonitrile scavenger is a substituted or unsubstituted sterically hindered aliphatic thiol group, a substituted or unsubstituted aromatic thiol, a substituted or unsubstituted aromatic hydroxyl group, a substituted or unsubstituted secondary aliphatic amine, a substituted or unsubstituted sterically hindered primary aliphatic amine or a substituted or unsubstituted primary or secondary aromatic amine.
35. The method of Claim 34, wherein the acrylonitrile scavenger is *t*-butylamine.
36. The method of Claim 25, wherein the basic solution comprises an alkali metal hydroxide or an alkaline earth metal hydroxide.
- 20 37. The method of Claim 25, wherein the basic solution is an ammonium hydroxide solution.

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38. The method of Claim 37, wherein the temperature of the ammonium hydroxide solution is about 20°C to about 100°C.
39. The method of Claim 38, wherein the temperature of the ammonium hydroxide solution is about 45°C to about 65°C.
- 5 40. The method of Claim 39, wherein the temperature of the ammonium hydroxide solution is about 55°C.
41. The method of Claim 37, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 0.5 hours to about 48 hours.
- 10 42. The method of Claim 41, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 6 hours to about 16 hours.
43. The method of Claim 25, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at  
15 least about 5%.
44. The method of Claim 43, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 25%.
45. The method of Claim 44, wherein the combined percentage of nucleobases  
20 which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 50%.

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46. The method of Claim 45, wherein all the nucleobases are thymine or guanine.
47. A method of substantially preventing modification of a synthetic oligonucleotide or oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group from the oligonucleotide or an oligonucleotide analog, comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising *t*-butylamine and an organic solvent selected from the group consisting of a haloalkane, an ester, an alcohol or dimethyl formamide under conditions sufficient to remove at least one nucleobase protecting group and at least one  $\beta$ -cyanoethyl protecting group.
48. A method of substantially preventing modification of a synthetic oligonucleotide or oligonucleotide analog during cleavage of at least one  $\beta$ -cyanoethyl protecting group and at least one nucleobase protecting group from the oligonucleotide or an oligonucleotide analog, comprising the step of contacting a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with an ammonium hydroxide solution containing *t*-butylamine under conditions sufficient to remove at least one nucleobase protecting group and at least one  $\beta$ -cyanoethyl protecting group.
49. The method of Claim 48, further comprising the step of contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising an organic solvent and at least one acrylonitrile scavenger prior to contacting the oligonucleotide or oligonucleotide analog with the aqueous basic solution.



50. The method of Claim 49, wherein the organic solvent is selected from the group consisting of pyridine, tetrahydrofuran, acetonitrile, a haloalkane, an esters, an alcohol and dimethyl formamide.
51. The method of Claim 50, wherein the organic solvent is pyridine or acetonitrile.
- 5 52. The method of Claim 48, wherein a the  $\beta$ -cyanoethyl protecting groups and the nucleobase protecting groups are removed from a phosphate triester oligonucleotide.
53. The method of Claim 48, wherein a the  $\beta$ -cyanoethyl protecting groups and the nucleobase protecting groups are removed from a phosphorothioate  
10 oligonucleotide analog.
54. The method of Claim 48, wherein the synthetic oligonucleotide or oligonucleotide analog is attached to a solid support by a covalent bond.
55. The method of Claim 54, wherein the solid support is controlled-pore glass, polystyrene or poly(acrylamide).
- 15 56. The method of Claim 55, wherein the oligonucleotide or oligonucleotide analog is cleaved from the solid support by contact with a basic solution having at least one acrylonitrile scavenger.
57. The method of Claim 48, wherein the temperature of the ammonium hydroxide solution is about 20°C to about 100°C.

58. The method of Claim 57, wherein the temperature of the ammonium hydroxide solution is about 45°C to about 65°C.
59. The method of Claim 58, wherein the temperature of the ammonium hydroxide solution is about 55°C.
- 5 60. The method of Claim 48, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 0.5 hours to about 48 hours.
61. The method of Claim 60, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for  
10 about 6 hours to about 16 hours.
62. The method of Claim 48, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 5%.
63. The method of Claim 62, wherein the combined percentage of nucleobases  
15 which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 25%.
64. The method of Claim 63, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 50%.
- 20 65. The method of Claim 64, wherein all the nucleobases are thymine or guanine.

66. A method of producing an oligonucleotide or oligonucleotide analog, wherein modification of the oligonucleotide or oligonucleotide analog during removal of the  $\beta$ -cyanoethyl protecting group is substantially prevented, comprising the steps of:
- 5 a) synthesizing an oligonucleotide or oligonucleotide analog having at least one  $\beta$ -cyanoethyl protecting group; and
- b) contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising at least one acrylonitrile scavenger and an organic solvent selected from the group consisting of a haloalkane, an ester, an alcohol or dimethyl formamide under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group, provided that when the acrylonitrile scavenger comprises a primary aliphatic amine or a primary aliphatic thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered, whereby the  $\beta$ -cyanoethyl protecting group is removed without substantially modifying the oligonucleotide or oligonucleotide analog.
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67. A method of producing an oligonucleotide or oligonucleotide analog, wherein modification of the oligonucleotide or oligonucleotide analog during removal of the  $\beta$ -cyanoethyl protecting group is substantially prevented, comprising the steps of:
- 20 a) synthesizing an oligonucleotide or oligonucleotide analog having at least one  $\beta$ -cyanoethyl protecting group; and
- b) contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with an aqueous basic solution having at least one acrylonitrile scavenger under conditions sufficient to remove at least one  $\beta$ -cyanoethyl protecting group, provided that when the acrylonitrile scavenger comprises a primary aliphatic amine or a primary aliphatic
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thiol, the primary aliphatic amine or the primary aliphatic thiol is sterically hindered, whereby the  $\beta$ -cyanoethyl protecting group is removed without substantially modifying the oligonucleotide or oligonucleotide analog.

- 5 68. The method of Claim 67, further comprising the step of contacting the  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog with a solution comprising an organic solvent and at least one acrylonitrile scavenger prior to contacting the oligonucleotide or oligonucleotide analog with the aqueous basic solution.
- 10 69. The method of Claim 68, wherein the organic solvent is selected from the group consisting of pyridine, tetrahydrofuran, acetonitrile, a haloalkane, an esters, an alcohol and dimethyl formamide.
70. The method of Claim 69, wherein the acrylonitrile scavenger is *t*-butylamine and the organic solvent is pyridine or acetonitrile.
- 15 71. The method of Claim 67, wherein the synthetic oligonucleotide or oligonucleotide analog is synthesized using phosphoramidite chemistry.
72. The method of Claim 71, wherein at least one nucleobase protecting group is cleaved when the synthetic oligonucleotide or oligonucleotide analog is contacted with the aqueous basic solution having at least one acrylonitrile  
20 scavenger.
73. The method of Claim 67, wherein the oligonucleotide produced is a phosphate diester oligonucleotide.

74. The method of Claim 67, wherein a the oligonucleotide analog produced is a phosphorothioate oligonucleotide analog.
75. The method of Claim 67, wherein the synthetic oligonucleotide or oligonucleotide analog is attached to a solid support by a covalent bond.
- 5 76. The method of Claim 75, wherein the solid support is controlled-pore glass, polystyrene or poly(acrylamide).
77. The method of Claim 76, wherein the oligonucleotide or oligonucleotide analog is cleaved from the solid support by contact with a basic solution having at least one acrylonitrile scavenger.
- 10 78. The method of Claim 67, wherein the acrylonitrile scavenger is a substituted or unsubstituted sterically hindered aliphatic thiol group, a substituted or unsubstituted aromatic thiol, a substituted or unsubstituted aromatic hydroxyl group, a substituted or unsubstituted secondary aliphatic amine, a substituted or unsubstituted sterically hindered primary aliphatic amine or a substituted or  
15 unsubstituted primary or secondary aromatic amine.
79. The method of Claim 78, wherein the acrylonitrile scavenger is *t*-butylamine.
80. The method of Claim 67, wherein the basic solution comprises an alkali metal hydroxide or an alkaline earth metal hydroxide.
81. The method of Claim 67, wherein the basic solution is an ammonium hydroxide  
20 solution.

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91. The method of Claim 87, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 0.5 hours to about 48 hours.
- 5 92. The method of Claim 91, wherein the synthetic oligonucleotide or oligonucleotide analog is contacted with the ammonium hydroxide solution for about 6 hours to about 16 hours.
93. The method of Claim 72, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 5%.
- 10 94. The method of Claim 93, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 25%.
- 15 95. The method of Claim 94, wherein the combined percentage of nucleobases which are thymine and guanine in oligonucleotide or oligonucleotide analog is at least about 50%.
96. The method of Claim 95, wherein all the nucleobases are thymine or guanine.
- 20 97. A method of preparing an oligonucleotide or an oligonucleotide analog comprising removing at least one  $\beta$ -cyanoethyl group from a  $\beta$ -cyanoethyl protected oligonucleotide or oligonucleotide analog using the method of any one of Claims 1-65.

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